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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P045575PCT	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/NL 03/00818	International filing date *(day/month/year)* 20.11.2003	Priority date *(day/month/year)* 20.11.2002
International Patent Classification (IPC) or both national classification and IPC C12P3/00		
Applicant WAGENINGEN UNIVERSITY et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
16.06.2004	19.11.2004
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Schmitz, T Telephone No. +31 70 340-4494

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/NL 03/00818

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-12 as originally filed

Claims, Numbers

1-13 received on 05.11.2004 with letter of 05.11.2004

Drawings, Sheets

1/2-2/2 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-12
	No:	Claims	13
Inventive step (IS)	Yes:	Claims	1-12
	No:	Claims	13
Industrial applicability (IA)	Yes:	Claims	1-13
	No:	Claims	-

2. Citations and explanations

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: BOETIUS ANTJE ET AL: "A marine microbial consortium apparently mediating anaerobic oxidation of methane." NATURE (LONDON), vol. 407, no. 6804, 2000, pages 623-626, XP002236081 ISSN: 0028-0836

D2: BALK MELIKE ET AL: "Thermotoga lettingae sp. nov., a novel thermophilic, methanol-degrading bacterium isolated from a thermophilic anaerobic reactor." INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY. ENGLAND JUL 2002, vol. 52, no. Pt 4, July 2002 (2002-07), pages 1361-1368, XP008015281 ISSN: 1466-5026

D3: HOEHLER TORI M ET AL: "Field and laboratory studies of methane oxidation in an anoxic marine sediment: Evidence for a methanogen-sulfate reducer consortium." GLOBAL BIOGEOCHEMICAL CYCLES, vol. 8, no. 4, 1994, pages 451-463, XP008015376 ISSN: 0886-6236

D4: WO 02/06503 A (US ENERGY) 24 January 2002 (2002-01-24)

D5: NAUHAUS KATJA ET AL: "In vitro demonstration of anaerobic oxidation of methane coupled to sulphate reduction in sediment from a marine gas hydrate area." ENVIRONMENTAL MICROBIOLOGY. ENGLAND MAY 2002, vol. 4, no. 5, May 2002 (2002-05), pages 296-305, XP002236082 ISSN: 1462-2912

D6: VALENTINE D L ET AL: "Hydrogen production by methanogens under low-hydrogen conditions." ARCHIVES OF MICROBIOLOGY. GERMANY DEC 2000, vol. 174, no. 6, December 2000 (2000-12), pages 415-421, XP002236084 ISSN: 0302-8933

1. The document D1, which is considered to represent the closest prior art, discloses (Figures 1-3; Abstract, Equation 1) a process for anaerobically reducing sulphate to sulphide by an Archaea / Sulphate Reducing Bacteria (SRB) consortium at 4 °C. Sulphate has been proposed to be the terminal electron acceptor for anaerobic oxidation of methane (equation 1):

$$CH_4 + SO_4^{2-} \longrightarrow HCO^{3-} + HS^- + H_2O$$

The authors conclude from their experiments (p. 625, left column, lines 25-28), "that the process is a reversal of methane formation, involving methanogens and a sulfate reducing partner which effectively scavenges intermediates such as H_2 or

acetate".
The authors further conclude (p. 625, left column, lines 45-62) that H_2 formation would be the most favourable explanation.
Present claim 1 differs from D1 in that specific microorganisms (Thermotoga maritima and T. lettingae) were experimentally shown to be capable of producing Hydrogen from Methane.

The problem to be solved by the present application is therefore considered to be the provision of a process for the anaerobic oxidation of methane to produce hydrogen.

The solution proposed, namely the hydrogen production by anaerobic methane oxidation (reversed methanogenesis) by Thermotoga maritima and T. lettingae does involve an inventive step for the following reasons:
The document D1 does not guide the person skilled in the art to use T. maritima or T. lettingae for said process. Furthermore, until the filing date of the present application, no organism capable of reversed methanogenesis in pure culture was identified.

A consortium hypothesis, explaining anaerobic methane oxidation, in which methanogens are postulated to operate in reverse ("reversed methanogenesis") was first disclosed in 1994 in document D3. Since then, a number of publications failed to provide evidence in favour of the hypothesis, see for example D5 (page 296, right column, first paragraph; page 301, right column, first paragraph) and D6 (page 419, last paragraph - page 420, first paragraph).

In conclusion, by providing said process comprising specific organisms, the applicants have solved a technical problem which workers in the art have been attempting to solve for a long time, thereby fulfilling a long-felt need.

Finally, the document D4 discloses a method for hydrogen production involving various Thermotoga species, including T. maritima and T. napolitana. The document mentions several hydrocarbons, but remains silent about methane oxidation or methane as a substrate for hydrogen production.

In summary, the subject matter of claims 1-12 is new, involves an inventive step and meets the requirements of Article 33 PCT.

2. Independent claim 13 refers to a mere "mixed culture". It is not at present clear, what problem is solved by present claim 13. However, in as far as a problem can be identified, the following is noted:

Document D2, which is considered to represent the closest prior art for this part of the invention, discloses (lines 6-8 of abstract; page 1365, right column, first paragraph) a syntrophic culture of TMO^T (Thermotoga lettingae sp.) and Thermodesulfovibrio yellowstonii. The subject matter of claim 13 differs from this in that a different "mixed culture" is claimed.

The problem to be solved by present claim 13 may therefore be regarded as the provision of an alternative coculture.

The solution proposed, namely the provision of a further coculture comprising a Thermotogales species and a sulfate reducing species cannot be considered as involving an inventive step for the following reasons:
In view of the disclosure in document D2, the skilled person would regard it as one of several straightforward options to coculture said organisms. The skilled person would proceed without the use of inventive skill, using common knowledge only, with a reasonable expectation of success. In conclusion, the subject-matter of claim 13 does not involve an inventive step (Article 33(3) and 6 PCT)

Re Item VIII

Certain observations on the international application

3. Claims 1 and 6 do at present not seem to be supported by the description as required by Article 6 PCT, as their scope is broader than justified by the description and drawings. The reasons therefor are the following:
the examples showing hydrogen production by anaerobic methane oxidation are limited to Thermotoga maritima and Thermotoga lettingae. It does at present not appear justified to assume that all Thermotogales species are capable of producing hydrogen under said conditions.

Claims

1. A process for converting methane to produce hydrogen or hydrogen equivalents, *characterised* in that methane is subjected anaerobically to the activity of methane-oxidising bacteria of the order of the *Thermotogales*.

2. A process according to claim 1, wherein the methane-oxidising bacteria comprise a *Thermotoga* species.

3. A process according to claim 2, wherein the *Thermotoga* species comprises *T. maritima* or *T. lettingae*.

4. A process according to any one of claims 1-3, which is carried out at a temperature between 25 and 90°C.

5. A process according to any one of claims 1-4, which is carried out in the presence of thiosulphate.

6. A process for reducing chemical compounds by biological reduction using hydrogen equivalents, *characterised* in that the hydrogen equivalents are produced by subjecting methane to anaerobic methane-oxidising bacteria of the order of the *Thermotogales*.

7. A process according to claim 6, wherein sulphur compounds are reduced to sulphide using a sulphate-reducing species.

8. A process according to claim 7, wherein the sulphur compounds comprise sulphate and/or sulphite.

9. A process according to claim 7 or 8, wherein the anaerobic methane-oxidising species comprises a *Thermotoga, Thermosipho* or *Fervidobacterium* species.

10. A process according to claim 7 or 8, wherein the sulphate-reducing species comprises an *Archaeglobus, Desulfotomaculum, Desulforomonas, Desulfovibrio* or *Thermodesulfovibrio* species.

11. A process according to claim 6, wherein metals are reduced from a high valence state to a low-valence or zero-valence state.

12. A process according to any one of claims 6-11, wherein a temperature of between 25 and 90°C is used.

13. A mixed culture use, containing one ore more anaerobic methane-oxidising *Thermotogales* species, and one or more sulphate-reducing or metal reducing species, in particular a *Archaeglobus, Desulfotomaculum, Desulforomonas, Desulfovibrio* or *Thermodesulfovibrio* species.

AMENDED CLAIMS

[Received by the International Bureau on 13 April 2004 (13.04.04): original claim 13 amended; remaining claims unchanged; (2 pages)]

Claims

1. A process for converting methane to produce hydrogen or hydrogen equivalents, *characterised* in that methane is subjected anaerobically to the activity of methane-oxidising bacteria of the order of the *Thermotogales*.

2. A process according to claim 1, wherein the methane-oxidising bacteria comprise a *Thermotoga* species.

3. A process according to claim 2, wherein the *Thermotoga* species comprises *T. maritima* or *T. lettingae*.

4. A process according to any one of claims 1-3, which is carried out at a temperature between 25 and 90°C.

5. A process according to any one of claims 1-4, which is carried out in the presence of thiosulphate.

6. A process for reducing chemical compounds by biological reduction using hydrogen equivalents, *characterised* in that the hydrogen equivalents are produced by subjecting methane to anaerobic methane-oxidising bacteria of the order of the *Thermotogales*.

7. A process according to claim 6, wherein sulphur compounds are reduced to sulphide using a sulphate-reducing species.

8. A process according to claim 7, wherein the sulphur compounds comprise sulphate and/or sulphite.

9. A process according to claim 7 or 8, wherein the anaerobic methane-oxidising species comprises a *Thermotoga, Thermosipho* or *Fervidobacterium* species.

10. A process according to claim 7 or 8, wherein the sulphate-reducing species comprises an *Archaeglobus, Desulfotomaculum, Desulforomonas, Desulfovibrio* or *Thermodesulfovibrio* species.

11. A process according to claim 6, wherein metals are reduced from a high valence state to a low-valence or zero-valence state.

12. A process according to any one of claims 6-11, wherein a temperature of between 25 and 90°C is used.

13. A mixed culture, containing one ore more anaerobic methane-oxidising *Thermotogales* species, and one or more sulphate-reducing or metal reducing *Archaeglobus, Desulfotomaculum, Desulforomonas* or *Desulfovibrio* species.